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RESEARCH PAPER

Optimum conditions for pure culture of major ectomycorrhizal fungi obtained from *Pinus sylvestris* var. *mongolica* plantations in southeastern Keerqin sandy lands, China

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Abstract: The effects of medium, pH, water potential and temperature on the culture for three pure strains (*Lactarius deliciosus*, *Boletus edulis* and *Lactarius insulsus*) of ectomycorrhizal fungi from plantation forests of Mongolian pine (*Pinus sylvestris* var. *mongolica*) on sandy lands were observed to obtain the optimum conditions for the growth of ectomycorrhizal fungi. The results indicated that the three ectomycorrhizal fungi could grow well in the mediums containing natural components, such as vitamin, pine juice and yeast powder. pH had a slight effect on the growth of the three ectomycorrhizal fungi, and the optimum pH values were 6.0 for *L. deliciosus*, 5.0 for *B. edulis*, respectively. However, *L. insulsus* had a wide pH range, and it grew better than the other two strains in neutral and light alkalescent mediums. Water potential (produced by Polyethylene Glycol, PEG) had significant effects on the ecological adaptability for the tested three fungi strains. All of the three stains grow better at lower PEG concentration (100 g PEG·kg^{-1} H_2O). The best water potential was 10% PEG concentration for all of the three stains. Temperatures, especially high temperatures induced the fungi death. The optimum temperature for the growth of ectomycorrhizal fungi was 25–28℃ for all of the three stains.

Keywords: Mongolian pine (*Pinus sylvestris* var. *mongolica*); ectomycorrhizal fungi; medium; pH; water potential; temperature

Introduction

The ectomycorrhizal symbiosis (ECM) is ubiquitous in roots of many plant families such as Pinaceae, Fagaceae and Betulaceae (Smith and Read 1997). Ectomycorrhizal fungi play an important role in the development of forest ecosystem, because the dominant trees in most of the world's temperate and boreal forests and in large areas of tropical and subtropical forests are ectomycorrhizal dependent (Allen 1991). It is believed that the ECM symbiosis is an adaptation to ecosystem with low mineral nutrient availability (Smith and Read 1997). The ECM system may enhance the host's nutrient status, not only by increasing the nutrient-absorbing surface area through the extraradical mycelium, but also by accessing nutrients not available to plants. ECM can also protect trees against pathogenic infections and improve the structure of root systems. Additionally, the ECM fungi may offer the host physical protection against drought, herbivory and pathogens, and ameliorate the deleterious effects of heavy metals on host plant (Ahonen-Jonnarth 2000) etc.

As a valuable conifer tree species (cold–resistant, drought-resistant and fast-growing), Mongolian pine (*Pinus sylvestris* var. *mongolica*) has been broadly introduced to the sandy lands in arid and semi-arid areas in "Three north" regions (North, northwest and northeast of China) of China. However, the plantations of Mongolian pine on sandy lands have declined at different degrees since early 1990s (Zhu et al. 2003; Chen et al. 2004; Zhu et al. 2005). Meanwhile, the natural Mongolian pine forest, which locates in Daxinganling Mountain and Hulunbeier sandy plain of China (N46°30′–53°59′, E118°00′–130°08′) (Zhu et al. 2003), still grows healthily at the same age of the plantations. There have been many assumptions reported to explain the causes of the decline (Jiao 2001). But till now, no specific theories could interpret it successfully. ECM are common in these plantations and are probably ecologically important in nutrient cycling, but environmental factors such as soil characteristics and climate characteristics probably have influences on mycorrhizal formation and fungal development (Smith and Read 1997). Johansson (2002) suggested that pH had an influence on ECM communities. Temperature and precipitation are the major

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factors that influence the occurrence of mycorrhizal fungi (Zhang et al. 1996).

In Zhanggutai, Zhangwu County, Liaoning Province, southeastern Keerqin sandy lands, China, the climate characteristics can be described as a long period of drought and high temperature in summer. The sandy soil can not keep water when rain falls, and the soil fertility is poor. Such environmental conditions must have a great influence on the growth and diversity of the fungal strains.

The present study is to explore the optimum ranges of the growth for the ectomycorrhizal fungi from Mongolian pine plantations on sandy lands in four aspects, i.e., mediums, pH, water potential and temperature. The results of present work may give some references to the decline of Mongolian pine plantation from the view of ECM, and provide some suggestions for the afforestation and management of large-scale Mongolian pine plantation forests through the way of Mycorrhizae on sandy lands.

Materials and methods

Study site

The fruiting bodies were collected at Zhanggutai, Zhangwu County, Liaoning Province, southeastern Keerqin sandy lands (N42°39.7′, E122°33.6′), China. The experimental area belongs to arid-sub-humid region (Fig. 1). The mean annual temperature is 5.7°C, and the extreme lowest and highest temperatures are -29.5°C and 37.2°C, respectively. The altitude of the site is 247.6 m. Annual precipitation is 450.0 mm, and the extreme lowest and highest annual precipitations are separately 224.8 mm and 661.3 mm. Annual evaporation is about 1700.0 mm. The free frost period is about 154 d (Wu et al. 2002). Major soil types are aeolian sandy soil and meadow soil (belong to Semiaripsamment taxonomic group). Average groundwater table is 5.3 m. The dominant tree species of plantation is Mongolian pine (Chen et al. 2004; Zhu et al. 2005). The ectomycorrhizal fungi strains were collected from study site. The culture experiment was conducted at Institute of Applied Ecology, Chinese Academy of Science.

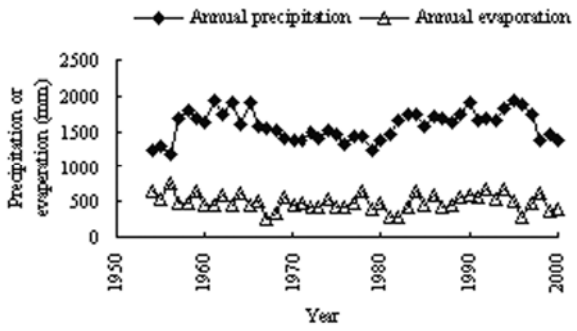

Fig. 1 Annual precipitation and evaporation from 1954 to 2000 in the investigated area

Materials

Fruiting bodies were surveyed at 3-week intervals from the beginning of July to the end of October in Mongolian pine plantations. Mycelia were obtained from fresh fruit bodies and isolated in solid potato-destrose-marmite agar (PDA) medium (Guo and Bi 1989). Some strains were gained, and three of them were selected in the experiment, i.e., *Lactarius insulsus*, *Boletus edulis* and *Lactarius deliciosus*. Isolates were cultured in modified Melin-Norkrans solid medium (Marx 1969), hereafter referred to as MMN two weeks.

Medium

In order to find appropriate medium for the strains, different mediums were selected to test appropriate nutrient component for the strains. Uniform inoculum plugs (4 mm in diameter) were taken from the colony edge and sub-cultured in Petri dishes with MMN. A minimum of three replications were maintained for each treatment.

Ten sorts of medium were used in this experiment. The names and components of the mediums were as follows.

Ⅰ potato juice iron-magnesium agar: 20% potato juice 1000 mL, glucose 20 g, $FeSO_4$ 0.01g, $MgSO_4{\cdot}7H_2O$ 0.01 g, agar 20 g, pH=5.5;

Ⅱ pine juice potato juice iron-magnesium agar: the same as Ⅰ but adding 20% pine juice;

Ⅲ potato destrose agar: 20% potato juice 1000 mL, glucose 20g, KH_2PO_4 3 g, $MgSO_4{\cdot}7H_2O$ 1.5 g, vitamine B_1 tittle, agar 20 g, pH=6.0;.

Ⅳ pine juice potato destrose agar: the same as Ⅲ but adding 20% pine juice;

Ⅴ ammonium chloride (NH_4Cl) glucose agar: (generally used for the culture of mycorrhizae) KH_2PO_4 1 g, NH_4Cl 0.5 g, glucose 0.5 g, $CaCl_2$ 0.1 g, NaCl 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.3 g, $FeCl_3$ 0.01 g, agar 20 g, distilled water 1000 mL;

Ⅵ pine juice ammonium chloride (NH_4Cl) glucose agar: the same as Ⅴ but adding 20% pine juice;

Ⅶ sucrose agar: sucrose 2.5 g, $(NH_4)_2HPO_4$ 0.25 g, $CaCl_2$ 0.05 g, $MgSO_4{\cdot}7H_2O$ 0.25 g, NaCl 0.25 g, $FeCl_3$(1%) 2 mL, KH_2PO_4 0.5 g, vitamin B_1 25 mg, agar 20 g, distilled water 1000 mL, pH=5.5-5.7;

Ⅷ glucose yeast agar: glucose 20 g, yeast powder 5 g, agar 20 g, distilled water 1000 mL, pH=5.2;

Ⅸ Heli medium: glucose 0.5 g, KH_2PO_4 1 g, $CaCl_2$ 0.1 g, $MgSO_4{\cdot}7H_2O$ 0.3 g, NH_4Cl 0.5 g, $FeCl_3$ 0.01 g, NaCl 0.1 g, agar 20 g, distilled water 1000 mL, pH=6.0;

Ⅹ asparagines agar (Zhou et al. 1983): (generally used for the culture of boletes) starch 20 g, KH_2PO_4 0.5 g, asparagine 1.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, agar 20 g, distilled water 1000 mL, pH=5.5.

pH

Isolates of *Lactarius insulsus*, *Boletus edulis* and *Lactarius deliciosus* were inoculated in solid and liquid MMN. Three treatments were set up to lower the pH of the MMN: 1) concentrated H_2SO_4, 2) concentrated HNO_3, and 3) a 1:1 (volume/volume) mixture of concentrated H_2SO_4 and HNO_3. We also used NaOH 1 $mol{\cdot}L^{-1}$ to increase pH, where no acids were added (Sánchex et al. 2001). The pH values in all treatments varied from 4 to 9 in one unit interval (n=4). Three replications were maintained for

each treatment.

Inoculated plates were incubated in the dark at 25°C for 15 days. At the solid MMN, we measured the colony diameters along the same orientation. Through centrifugaling and filtering the liquid MMN, we obtained the mycelial biomass, and then oven-drying them at 105℃ until the oven-dry weight not varied.

Water potential

Isolates of mycelial plugs were sub-cultured in Petri dishes containing liquid MMN with different water potentials. In this experiment, we used Polyethylene Glycol 6000 (PEG-6000) to produce a range of water potential in liquid MMN. 25mL milliliters MMN were dissolved PEG-6000 to reach the following concentrations: 0, 100, 200, 250 and 300 g PEG·kg^{-1} H_2O, with water potentials of -0.02, -0.20, -0.75, -1.35 and -1.53 MPa, respectively. Those solutions were measured using a Wescor Inc USA.

A sterilized grit support was placed in the Petri dishes with the liquid medium just covering the grit, and a fibre filter was placed on the grit with inoculum plug on the filter. Each dishes contained 25 mL MMN (Sánchex et al. 2001). To determine if PEG-6000 was a suitable carbon source in the experiment, the strains were grown on MMN with PEG-6000 as carbon sources substituted for glucose. The cultures were incubated at 25°C for 15 days. The colony diameter was measured at the end of the experiment. Three replications were maintained for each treatment.

Temperature

In this treatment, cultures of ectomycorrhizal fungi in solid MMN were incubated in the dark at 5, 10, 20, 25, 28, 30, 37, 40°C. After 15 days, colony diameters were measured. Three replications were maintained for each treatment.

Statistic Analysis

Analysis of variance was used to determine the significant differences among mean values between treatments, and Duncan's multiple range test was used for significant differences between treatments. The software of SPSS (Version 11.5) was applied.

Results

Different mediums for ECM species

The growth of ECM needs high nutrition. *L. insulsus* grew well in potato juice iron-magnesium agar, pine juice potato juice iron-magnesium agar, potato destrose agar, pine juice ammonium chloride (NH_4Cl) glucose agar and glucose yeast agar. *B. edulis* grew well in potato juice iron-magnesium agar, potato destrose agar, pine juice ammonium chloride (NH_4Cl) glucose agar and glucose yeast agar. *L. deliciosus* grew well in pine juice potato juice iron-magnesium agar, potato destrose agar, pine juice potato destrose agar and Heli medium (Table 1). All of the strains selected grew well in the mediums which contain vitamin or yeast powder, and grew slowly in the mediums which mostly are inorganic chemistry salts.

Table 1. There ECM species culture speed on different medium

Strain	Number of medium									
	I	II	III	IV	V	VI	VII	VIII	IX	X
L. deliciosus	-	+	+	+	-	-	-	-	+	-
B. edulis	+++	++	+++	++	+++	++	++	++	+++	+++
L. insulsus	++++	++++	++++	+++	+++	++++	+++	++++	+++	+++

Note: "++++" means the colony diameters were more than 70 mm; "+++" means the colony diameters were between 41 mm and 69 mm; "++" means the colony diameters were between 30 mm and 40 mm; "+" means the colony diameters were between 15 mm and 30 mm; "-" means the colony diameters were lower than 15 mm.

Effects of pH on the growth of the isolates

The optimum pH values for isolates were not homology for the three fungi species. For *L. deliciosus* the optimum pH value was 6.0, and it obtained the largest diameter and biomass. *L. insulsus* had the largest colony diameter at pH 6.0, and its dry weight was the largest at pH 7.0. However, *B. edulis* had the largest colony diameter and dry weight at pH 5.0 Within the pH interval 4–9 trial setting, isolates could grow, except that *L. deliciosus* did not develop at pH 8 and 9.

Although there were some differences in diameter growth, the three fungi species showed a similar pattern along pH values in the three treatments (Table 2). In the same treatment, *L. deliciosus* showed the smallest diameter. *L. insulsus* showed the greatest colony diameter when pH was modified with H_2SO_4. For *L. deliciosus,* the diameter showed significant differences between different pH values (Table 2).

Table 2. Colony diameters (mm) and dry weights (mg) (±: standard errors) of the different ectomycorrhizal species at different pH values for the media modified with H_2SO_4, HNO_3 and H_2SO_4+ HNO_3.

	Lactarius deliciosus					
pH	colony diameters(mm)			dry weight(mg)		
	H_2SO_4	HNO_3	H_2SO_4+HNO_3	H_2SO_4	HNO_3	H_2SO_4+HNO_3
4	19.1±0.1^a	18.5±0.3^c	16.1±0.1^b	107.7±4.0^a	100±.5^a	103±0.6^a
5	17.1±0.1^c	25.1±0.1^b	24.4±0.2^a	119.33±0.9^b	106±.6^c	115.7±3.8^{ab}
6	40.6±0.2^b	43.3±0.2^d	43.6±0.3^d	152±0.58^c	133±.1^b	115.7±8.1^a
	Boletus edulis					
pH	colony diameters(mm)			dry weight(mg)		
	H_2SO_4	HNO_3	H_2SO_4+HNO_3	H_2SO_4	HNO_3	H_2SO_4+HNO_3
4	44.2±1.2^a	42.5±3. 8^a	44.5±1.^{1a}	60.67±3.71^a	72.7±5^{ac}	44.3±1.2^b
5	55.8±1.7^b	40.2±1.4^a	59.0±4.4^b	60.33±4.18^a	84. 7±.1^c	62.3±4.1^{ac}
6	52.5±1^c	44.3±3^a	54.9±1.9^{bc}	66±2.08^{ac}	72.3±.2^{ac}	60±4.6^c
	Lactarius insulsus					
pH	colony diameters(mm)			dry weight(mg)		
	H_2SO_4	HNO_3	H_2SO_4+HNO_3	H_2SO_4	HNO_3	H_2SO_4+HNO_3
4	63.9±3.0^a	51.0±1^b	50.0±0.3^b	55.7±9.8^a	72.7±0.9^a	37±15.72^a
5	76.3±8.8^c	61.6±1.1^a	78.5±1.3^c	54±2.1^{ab}	79±1.7^b	39.3±18.6^{ab}
6	85.7±1.6^c	63.0±4.6^a	82.1±3.8^c	61.7±6.2^a	94.3±4.7^c	51±2.9^a

Note: Data with different letters indicated the significant difference at p<0.05 according to Duncan's multiple range test within the same treatment.

Regarding fungal biomass, *L. insulsus* showed the greatest dry weights when grown on media with NaOH (Table 3). *B. edulis* did not show significant differences between treatments except

modified pH using H_2SO_4+HNO_3 at pH 4. *B. edulis* and *L. insulsus* showed lower dry weight on H_2SO_4 than that on HNO_3, but no significant differences were observed for these treatments. Only the dry weight of *L. deliciosus* showed significant differences between different acids at pH 6.

For most of isolates, changes on medium pH were influenced by treatment and initial pH. All of the species could cause the decrease of the medium pH from the beginning to the end of the experiment, except that *L. insulsus* increased the medium pH when modified by HNO_3 and H_2SO_4+ HNO_3 (Fig. 2). *B. edulis* and *L. deliciosus* induced a decrease of the initial pH on medium of three treatments. For *B. edulis*, the largest changes in initial pH were observed in the HNO_3 treatments. The isolates produced greater decrease of the initial pH on medium modified with NaOH (Fig. 2).

Table 3. Colony diameters (mm) and dry weights (mg) (±standard errors) of the different ectomycorrhizal species at different pH values for the media modified with NaOH 1 mol·L^{-1}

pH	*Lactarius deliciosus*		*Lactarius insulsus*	
	colony diameters(mm)	dry weight (mg)	colony diameters(mm)	dry weight (mg)
7	16.3±0.06^a	126.33±1.76	56.1±2.0^a	114±7.6^a
8	4±0^b	0	77.9±8.4^b	56±4.5^b
9	4±0^b	0	62.6±7.5^a	68.7±15.0^{ab}
pH	*Boletus edulis*			
	colony diameters(mm)	dry weight(mg)		
7	44.0±1.2^a	54.3±5.4^a		
8	43.6±0^a	53±4.0^a		
9	40.0±0^b	59.3±1.5^a		

Note: Data with different letters indicated the significant difference at p =0.05 according to Duncan's multiple range test within the same treatment.

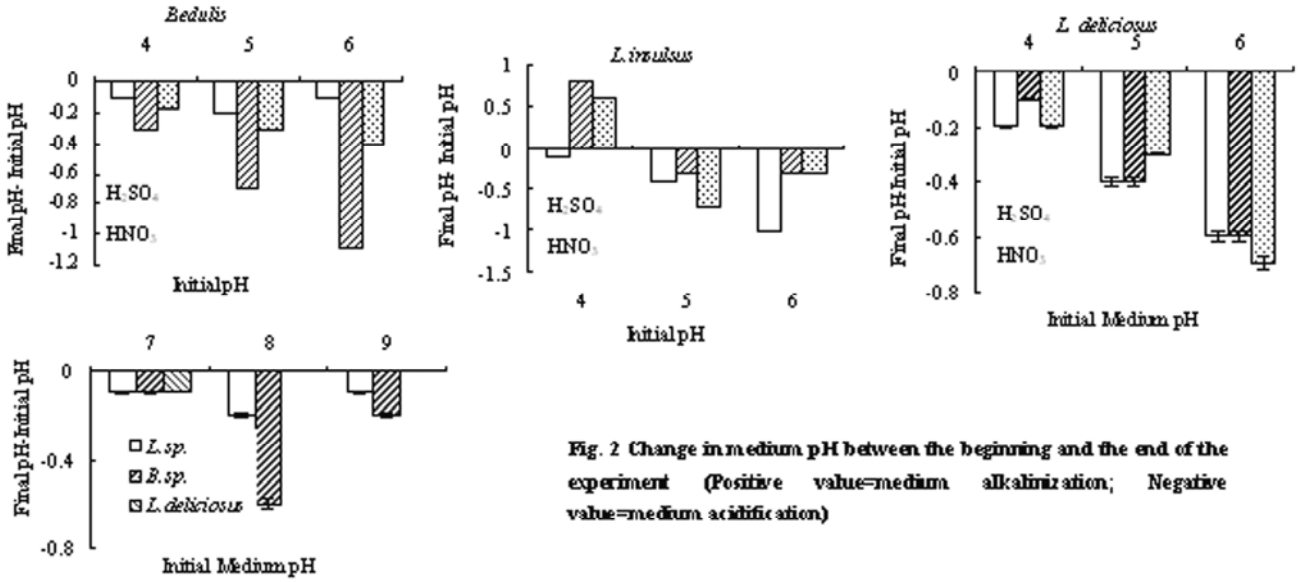

Fig. 2 Change in medium pH between the beginning and the end of the experiment (Positive value=medium alkalinization; Negative value=medium acidification)

Effects of water potential on the growth of the isolates

At highest water stress (300 g PEG·kg^{-1} H_2O, -1.53 MPa), all of the isolates could not grow. The optimum PEG concentration for the isolates were 100 g PEG·kg^{-1} H_2O (-0.20 MPa). This indicated that the isolates studied could tolerate low water stress. At the higher PEG concentrations, e.g. 200 and 250 g PEG·kg^{-1} H_2O (-0.75 and -1.35 MPa, respectively), *L. insulsus* had the greatest diameter. At low water stress (0 and 100 g PEG·kg^{-1} H_2O, water potential was -0.02 and -0.20 MPa, respectively), there is no significant difference between *L. insulsus* and *B. edulis* on colony diameter. Significant differences of fungal diameters at the high water stresses (250 and 300 g PEG·kg^{-1} H_2O, water potential was -1.35 and -1.53 MPa, respectively) between fungi species were found. *L. deliciosus* had the lowest colony diameter among the fungi species studied (Fig. 3).

Colony diameter of the isolates showed no change with PEG-6000 as carbon sources in the substrate, indicating that PEG-6000 was unsuitable carbon sources for the three fungi species.

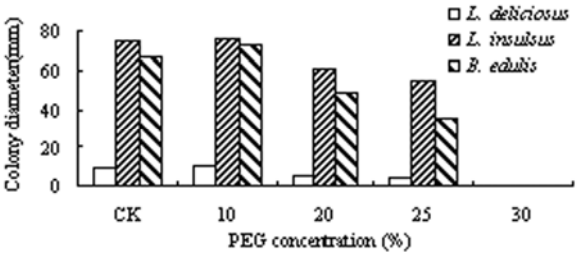

Fig. 3 Diameter growth response of ectomycorrhizal fungi to increasing PEG concentrations.

Effects of temperature on the growth of the isolates

Temperature had significantly effect on the growth of ectomycorrhizal fungi. The optimum temperature range for the growth of ectomycorrhizal fungi was 25–28°C (Fig. 4). *L. insulus* and *B. edulis* grew best at 25°C, and *L. deliciosus* grew best at 28°C *L. insulsus* and *B. edulis* could grow at 37℃, while *L. delicious* showed no colony diameter at this temperature. *L. delicious*

could not grow at 10°C, while the other two strains grew well at this temperature. The colony diameters for all the isolates were significantly smaller at 20°C than at the 25°C. This may suggest that even a small decrease in temperature has a negative effect on fungal growth. At 5°C and 40°C, all isolates showed no development.

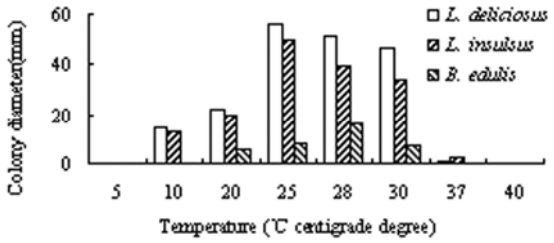

Fig. 4 Diameter growth response of ectomycorrhizal fungi to different temperatures.

Discussion

Pure culture of many fruiting bodies could not be obtained in some kinds of mediums, and the growth of ectomycorrhizal fungi needs peculiar nutrition (Zhou et al. 1983). Ectomycorrhizal fungi could grow better in the medium which contained natural components (Han et al. 1993), such as pine juice and vitamin. Maybe, those components have similarity to nature forest of their growth. The similar results were observed in this experiment.

In general, diametral growth and production of ectomycorrhizal fungi in pure culture were greatly influenced by changes in medium pH. Sánchex et al. (2001) reported that growth responses to various levels of acidity differed markedly among species. *B. edulis* and *L. insulsus* had vigorous growth at all pH levels. The optimum pH values for the two ectomycorrhizal fungi were 5.0–6.0, which verified the conclusion by Han et al. (1993) that mycorrhizal fungi favor acidic conditions. *L. deliciosus* and *B. edulis* produced the largest colony diameter and biomass, which indicated their capabilities to grow in acid substrates. *L. insulsus* isolates were tolerant to a neutral or slightly basic pH. This agrees with the results of Zhao and Guo (1989), which reported that *Suillus grevillei* grew better in the neutral pH conditions.

For the three fungi species, biomass production and colony diameter were not correlated to each other, because colony diameter reflected the capability of planar growth, but biomass reflected the three-dimensional growth (Zhao and Guo 1989). The colony diameter, reflecting the growth rates in vitro, formed a large quantity of aerial mycelia which made up lax hyphae, corresponding low dry weight. If the colonies were small and dense, even submerged in the medium, the biomass may be larger (Jonbloed and Borst-Pauwels 1990).

Isolates changed the medium pH during culturing (Fig. 2). The medium pH decreased except *L. insulsus*, which when it was modified with HNO_3 and HNO_3+H_2SO_4. Generally, organic acids are produced and some selective ions are taken up during in vitro development, as a result, it leads to a reduction in medium pH. However, some of isolates increased the medium pH. This may be explained that some OH^- are released to the medium after uptake of NO_3^{1-} and SO_4^{2-} according to Sánchez et al. (2001).

The growth response of the tested isolates to water potential showed two patterns. Type Ⅰ, growth increased up to certain stress levels, beyond which growth decrease (Fig. 3). Many ectomycorrhizal fungi exhibited such pattern (Zhao and Guo 1989). In our study, *L. deliciosus* and *B. edulis* showed this pattern. Type Ⅱ, growth did not exhibit a certain tendency, e.g., *L. insulsus* reached the larger colony diameters at 100 g PEG·kg^{-1} H_2O and 250 g PEG·kg^{-1} H_2O, while at control and 200 g PEG·kg^{-1} H_2O, its colony diameters were small. Machado and Braganca (1996) indicated that in vitro response to different water potentials may vary even among strains of the same species. Therefore, the strains from different areas may have different culture conditions. The origin of the ectomycorrhizal fungi may affect the ability to adapt in vitro to adverse water potentials. John Mexal and Reid (1973) reported that PEG could not be absorbed by ectomycorrhizal fungi as carbon source. Our study found similar results that the fungi species studied did not utilized PEG-6000 as carbon source.

The species and the number of ECM are influenced by many environmental factors, such as soil factors, climate conditions, and site factors (Xu et al. 2004). In natural ecological system, temperature is one of the most important factors to determine the occurrence of ECM, and influenced its growth and metabolization (Theodorou 1971). The ectomycorrhizal fungi usually can not grow well at some extreme temperatures (Bi et al. 1989). In Zhanggutai region, the fruiting bodies occurred in August and September, when the ground temperature ranges in 25–30°C. Based on the lab experiment result, the optimum temperature for the growth of isolates studied was 25–28°C (Fig. 4). This indicated that the highest richness of fungi species may occur when the temperature consisted with the optimum temperature. On the contrary, if the temperature is too high or too low, the formation of ectomycorrhizal fruits will be influenced. The soil surface temperature is too high in Zhanggutai region in summer, which may restrain survive of some ectomycorrhizal fungi.

Conclusions

For the tested three strains, they could grow better at some mediums which contain natural components, such as pine juice, potato juice, yeast power and vitamine. On the 10 mediums used, *L. insulsus* had the largest colony diameter, followed by *B. edulis*, and *L. delicious* had a small colony diameter. *L. insulsus* had the largest colony diameters in the medium with pH 4–9. It not only grew in acid medium, but also developed well in alkalescent medium. *L. insulsus* exhibited best in colony diameter for both PEG temperature treatments, and had largest dry weight among the three strains, and its optimum pH was 6.0. All the three stains grew best at lower PEG concentration (100 g PEG·kg^{-1} H_2O). This means low PEG concentration can stimulate ECM growth. The optimum temperature ranged 25–28°C for all the three strains.

Observing and identifying the optimum growth range of ectomycorrhizal fungi at lab, including different medium, pH, wa-

ter potential and temperature, may provide some references for the regeneration and renew of declined forest (Zhu et al. 2003). According to the experimental results, it can be concluded that *L. insulsus* has the most tolerance to alkalescent substrates and increasing water stresses, and this species can be applied for the forestation in some basic regions after the experiment of inoculation seedlings at laboratory. However, when ectomycorrhizal fungi is used to nursery inoculation programs, the environmental conditions and capability to form symbiotic system with plants must be taken into account (Zhao et al. 1989; Wu 1991; Xu et al. 2004). Therefore, the ectomycorrhizal fungi used in silviculture and the restoration of deteriorating forests must be chosen from the species which grow in the similar soil conditions (Wang et al. 2005).

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